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<input checked="" type="checkbox"/> Additional inventors are being named on page 2 attached hereto					
TITLE OF THE INVENTION (280 characters max)					
APRIL RECEPTOR AND USES THEREOF					
CORRESPONDENCE ADDRESS					
Direct all correspondence to:					
<input type="checkbox"/> Customer Number		<div>Place Customer Number Bar Code Label here</div>			
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ENCLOSED APPLICATION PARTS (check all that apply)					
<input checked="" type="checkbox"/>	Specification	Number of Pages	27		
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Respectfully submitted,

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cket No.: A083

APPLICATION  
FOR  
UNITED STATES PATENT

APRIL RECEPTOR AND USES THEREOF

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## APRIL RECEPTOR AND USES THEREOF

### FIELD OF THE INVENTION

The present invention relates generally to methods of treatment for cancer. The  
5 methods involve the administration of certain tumor necrosis factor (TNF) antagonists.

### BACKGROUND OF THE INVENTION

Members of the tumor-necrosis factor (TNF) family of cytokines are involved in an  
ever expanding array of critical biological functions. Each member of the TNF family acts  
10 by binding to one or more members of a parallel family of receptor proteins. These  
receptors in turn signal intracellularly to induce a wide range of physiological or  
developmental responses. Many of the receptor signals influence cell fate, and often  
trigger terminal differentiation. Examples of cellular differentiation include proliferation,  
maturation, migration, and death.

15 TNF family members are Type II membrane bound proteins, having a short  
intracellular N-terminal domain, a transmembrane domain, and the C-terminal receptor  
binding domains lying outside the cell surface. In some cases the extracellular portion of  
the protein is cleaved off, creating a secreted form of the cytokine. While the membrane  
bound proteins act locally, presumably through cell contact mediated interaction with their  
20 receptors, the secreted forms have the potential to circulate or diffuse, and therefore can act  
at distant sites. Both membrane bound and secreted forms exist as trimers, and are thought  
to transduce their signal to receptors by facilitating receptor clustering.

The TNF receptor protein family is characterized by having one or more cysteine  
rich extracellular domains. Each cysteine rich region creates a disulfide-bonded core  
25 domain, which contribute to the three dimensional structure that forms the ligand binding  
pocket. The receptors are Type I membrane bound proteins, in which the extracellular  
domain is encoded by the N-terminus, followed by a transmembrane domain and a C-  
terminal intracellular domain. The intracellular domain is responsible for receptor  
signaling. Some receptors contain an intracellular "death domain", which can signal cell  
30 apoptosis, and these can be strong inducers of cell death. Another class of receptors can  
weakly induce cell death, these appear to lack a death domain. A third class of receptors



do not induce cell death. All classes of receptors can signal cell proliferation or differentiation instead of death, depending on cell type or the occurrence of other signals.

A well studied example of the pluripotent nature of TNF family activity is the nominant member, TNF. TNF can exist as a membrane bound cytokine or can be cleaved and secreted. Both forms bind to the two TNF receptors, TNF-R55 and TNF-R75. Originally described on the basis on its' ability to directly kill tumor cells, TNF also controls a wide array of immune processes, including inducing acute inflammatory reactions, as well as maintaining lymphoid tissue homeostasis. Because of the dual role this cytokine can play in various pathological settings, both agonist and antagonist reagents have been developed as modifiers of disease. For example TNF and LT $\alpha$  (which also signals through the TNF receptors) have been used in treatment for cancers, especially those residing in peripheral sites, such as limb sarcomas. In this setting direct signaling by the cytokine through the receptor induces tumor cell death Aggarwal and Natarajan, 1996. Eur Cytokine Netw 7:93-124).

In immunological settings agents which block TNF receptor signaling (eg., anti-TNF mAb, soluble TNF-R fusion proteins) have been used to treat diseases like rheumatoid arthritis and inflammatory bowel disease. In these pathologies TNF is acting to induce cell proliferation and effector function, thereby exacerbating autoimmune disease, and in this setting blocking TNF binding to its receptor(s) has therapeutic benefit (Beutler, 1999. J Rheumatol 26 Suppl 57:16-21).

A more recently discovered ligand/receptor system appears amenable to similar manipulations. Lymphotoxin beta (LT $\beta$ ), a TNF family member which forms heterotrimers with LT $\alpha$ , bind to the LT $\beta$ -R. Some adenocarcinoma tumor cells which express LT $\beta$ -R can be killed or differentiated when treated with an agonistic anti-LT $\beta$ -R mAb (Browning et al., 1996. J Exp Med 183: 867-878). In immunological settings it has been shown that anti-LT $\beta$  mAb or soluble LT $\beta$ -R-Ig fusion protein can block the development of inflammatory bowel diseases, possibly by influencing dendritic cell and T cell interaction (Mackay et al., 1998. Gastroenterology 115:1464-1475).



The TRAIL system also has potential as a cancer therapy. TRAIL interacts with a number of membrane bound and soluble receptors. Two of these receptors, TRAIL-R1 and TRAIL R2 (also called DR4 and DR5), transmit death inducing signals to tumor cells but not to normal cells, which express additional TRAIL receptors which do not induce death. These additional receptors are thought to function as decoys. The use of soluble TRAIL to kill tumor cells relies on the selective expression of decoy receptors on normal but tumor tissue (Gura, 1997. Science 277: 768).

Tumor cells themselves often express a variety of decoy receptors that block immune recognition or effector functions. Indeed, some tumors overexpress TRAIL decoy receptors, apparently to avoid TRAIL mediated death (Sheikh et al., 1999. Oncogene 18: 4153-4159). This limits the utility of TRAIL as an anti-tumor agent in some settings. Similar observations have been made about a decoy receptor for FAS-L, which is overexpressed by lung and colon cancer cells (Pitti et al., 1998. Nature 396: 699-703), and for the IL-1 receptor antagonist (Mantovani et al., 1998. Ann N Y Acad Sci 840: 338-351). Decoy receptors are also employed by viral genomes to protect infected host cells from host defense mechanisms.

APRIL (A Proliferation Inducing Ligand) is a new member of the TNF family of proteins. APRIL expression and functional studies suggest that this protein is utilized by tumor cells to induce rapid proliferation. Tumor cell lines treated with soluble APRIL protein or transfected with APRIL cDNA grow rapidly in vitro. APRIL transfected cells implanted into immunodeficient mice grow rapidly as tumors. Finally, in human tumor cells, but not normal tissue, express high levels of APRIL messenger RNA. These observations suggest that APRIL binds to a receptor that is also expressed by tumor cells, setting up autocrine or paracrine tumor cell activation. In addition it is possible that APRIL acts in other disease settings, such that activating or blocking the APRIL pathway would have additional utility. For example, underexpression or overexpression of APRIL may play a role in developmental defects, since development is often characterized by the carefully controlled balance between cell proliferation and cell death. Similarly APRIL may act in cell proliferative diseases, such as occur in some autoimmune diseases (eg lupus) or in inflammatory diseases where cell populations expand rapidly (eg bacterial sepsis).





Based on the known utility of using agonists and antagonists of TNF and TNF receptor family members as disease modifiers, the APRIL pathway presents itself as an important target for drug development. This is particularly true for cancer therapy since tumor cells appear to produce and utilize APRIL to support their own growth, and are therefore unlikely to produce decoy receptors or other antagonists of the APRIL pathway. Thus the APRIL pathway is uniquely different from, for example, the TRAIL or FAS-L pathways, which can be thwarted by tumor decoy receptors.

Current treatments for cancer are inadequate for many tumor types, due to poor efficacy, low impact on survivorship, toxicity that causes severe side effects, or combinations thereof. Therefore there is a need to identify and develop additional methods for treating cancer growth which can provide efficacy without inducing severe side effects. Antagonists of the APRIL pathway, including anti-APRIL mAbs, anti-APRIL receptor mAbs, soluble APRIL receptor-Ig fusion proteins, natural antagonists, small molecule antagonists, and chemical, pharmaceutical, or other antagonists would thus be useful. To this end we have identified B cell mediated protein (BCM or BCMA) as a receptor for APRIL.

#### SUMMARY OF THE INVENTION

Applicants have found that BCMA is a receptor for the tumor-necrosis factor, APRIL. APRIL is the same molecule previously described in WO/99 12965, which is incorporated by reference herein. The APRIL receptor is referred to hereinafter as "APRIL-R". The present invention is directed to methods of treatment and pharmaceutical preparations for use in the treatment, of mammalian species having or at risk of having cancer. Such subjects include subjects already afflicted with cancer, or which have already received cancer therapy.

The methods and compositions of this invention capitalize in part upon the discovery that certain agents that are cancer therapeutic agents, defined herein as APRIL-R antagonists, including for example, anti-APRIL-R antibodies may be used in the treatment of subjects at risk, as defined herein, of cancer or the need for cancer treatment.



The cancer therapeutic agents of the invention may be administered by any route of administration which is compatible with the selected agent, and may be formulated with any pharmaceutically acceptable carrier appropriate to the route of administration. Preferred routes of administration are parenteral and, in particular, intravenous, intraperitoneal, and intracapsular. Treatments are also preferably conducted over an extended period on an outpatient basis. Daily dosages of the cancer therapeutic agents are expected to be in the range of about 0.01-1000  $\mu\text{g/kg}$  body weight, and more preferably about 10-300  $\mu\text{g/kg}$  body weight, although precise dosages will vary depending upon the particular cancer therapeutic agent employed and the particular subject's medical condition and history.

The treatments of the present invention are useful in eradicating a substantially clonal population (colony) of transformed cells from the body of a mammal, or to suppress or to attenuate the growth of the colony, which is most commonly referred to as a tumor. As such they are useful in prolonging the lives, and in maintaining the quality of life, of subjects at risk of, or already afflicted with cancer.



## BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows the nucleic acid sequence (SEQ ID NO:2) of a cDNA for murine APRIL-R and its derived amino acid sequence (SEQ ID NO:1) as mapped in vector pCCM213.10. Shown underlined is the myc epitope and the amino acids derived from FasL. The beginning of the APRIL coding sequence is indicated by arrows.

Figure 2 shows the nucleic acid sequence (SEQ ID NO: 4) and its derived amino acid sequence (SEQ ID NO:3) of FLAG-human APRIL construct for expression in mammalian cells. The map indicates the signal sequence (1-15); the FLAG epitope (AA 16-23) and the beginning of human APRIL coding sequence (32-end).

Figure 3 shows the nucleic acid sequence of pJST538, a plasmid encoding a full length human APRIL-R and its derived amino acid sequence.

Figure 4 shows binding of myc-murine APRIL to the murine B cell lymphoma line A20. 3 separate experiments show specific binding of APRIL to A20 cells compared to A) unstained cells and cells stained with R1532 only, B) cells stained with RANKL-L and R1532 and C) cells stained with APRIL and an irrelevant rabbit sera.

Figure 5 shows binding of myc-murine APRIL to the human B cell lymphoma line AJL. 2 separate experiments show specific binding of APRIL to A20 cells compared to A) unstained cells and cells stained with R1532 only, and cells stained with RANK-1 and R1532 and B) cells stained with APRIL and an irrelevant rabbit sera.

Figure 6 shows APRIL binding to A20 cells with Raji cells is competed using soluble BAFF protein or soluble BCMA-Ig protein.

Figure 7 shows binding of FLAG-human APRIL to various cell lines: A) A20 cells, B) HT29 cells, C) NIH3T3 cells. Specific binding is demonstrated using biotinylated anti-FLAG mAb M2 detection compared to binding seen with an irrelevant isotype control mAb or without addition of FLAG-APRIL.

Figure 8 shows immunoprecipitation of myc-mApril using BCMA-Fc fusion protein. Upper left panel show specific hBMCA-Fc/myc-mAPRIL and positive control OPG-Fc/Rank-1 immunoprecipitations, compared to upper right negative controls. Lower panels demonstrate that the amounts of protein loaded were equivalent.



Figure 9 shows an ELISA format experiments demonstrating that FLAG-h APRIL binds to hBCMA-fc fusion protein. Various receptor-Fc fusion proteins were coated onto the ELISA plates and bound with FLAG-tagged ligands. A) Detection of the bound ligands revealed that only APRIL and hBAFF specifically bind to hBCMA-Fc, but not hCD40-Fc. B) Dose titration showing that the ELISA signal detected after binding hAPRIL or hBAFF onto hBCMA-Fc coated plates is linearly dependent on the amount of protein added.

Figure 10 show an immunoprecipitation of FLAG-hAPRIL and FLAG-hBAFF by hBMCA-Fc fusion protein. Upper 4 panels show the equivalence of the protein loads in each immunoprecipitation, while the lower panels show that hAPRIL and hBAFF are immunoprecipitated by hBCMA-Fc but not hTRAIN-Fc.

Figure 11 show the BiaCore analysis of the binding of myc-mAPRIL, FLAG-hBAFF, and FLAG-mBAFF to hBMCA, hLTbeta receptor, or hTNF-R80 or blank showing specific binding only to hBCMA.

Figure 12 shows APRIL binding to BCMA infected cells. 293EBNA cells were transfected with a plasmid that expresses full length hBCMA. Cells were harvested 48 hours later using 5mM EDTA and stained with myc-nAPRIL. Panel A shows that the extent of staining is dose dependent. Panel B shows that the staining decreased to background level using a soluble BCMA-Ig protein.

## DETAILED DESCRIPTION

### Definitions

In order to more clearly and concisely point out the subject matter of the claimed invention, the following definitions are provided for specific terms used in the following written description and appended claims.

The invention will now be described with reference to the following detailed description of which the following definitions are included:

The term "APRIL receptor or APRIL-R" when used herein encompass native sequence APRIL-R and APRIL-R variants. The APRIL-R may be isolated from a variety of sources, such as from murine or human tissue types or from another source, or prepared by recombinant or synthetic methods. The term APRIL-R further refers to a polypeptide





which is capable of binding to the tumor necrosis family member, APRIL or homologs or fragments thereof. An example of an APRIL-R is BCMA

The term "BCMA" or "BCM" refers to the novel protein for B cell maturation as described in Gras et al. (1995), International Immunology, 7: 1093-1106, "BCMAp: an integral membrane protein in the golgi apparatus of human mature B lymphocytes"; Y. Laabi et al. (1992), EMBO J., 11, 3897-3904, "A new gene BCM on Chromosome 16 is fused to the interleukin 2 gene by a t(4;16) (q26;p13) translocation in a malignant T cell lymphoma".

A "native sequence APRIL-R" comprises a polypeptide having the same amino acid sequence as APRIL-R derived from nature. Such native sequence APRIL-R can be isolated from nature or can be produced by recombinant or synthetic means. The naturally-occurring truncated or secreted forms of the APRIL-R (e.g. soluble forms containing for instance, an extracellular domain sequence), naturally-occurring variant forms (e.g., alternatively spliced forms) and naturally-occurring allelic variants of the APRIL-R. In one embodiment of the invention, the native sequence APRIL-R is a mature or full-length native sequence APRIL-R polypeptide comprising amino acids 1 to 184 of SEQ ID NO: 1 or fragment thereof.

The "APRIL-R extracellular domain" or "APRIL-R ECD" refers to a form of APRIL-R which is essentially free of transmembrane and cytoplasmic domains of APRIL-R. Ordinarily, APRIL-R extracellular domain will have less than 1% of such transmembrane and cytoplasmic domains and will preferably have less than 0.5% of such domains. Optionally, APRIL-R ECD will comprise amino acid residues 8 to 41 of SEQ ID NO:1. It will be understood by the skilled artisan that the transmembrane domain identified for the APRIL-R polypeptide of the present invention is identified pursuant to criteria routinely employed in the art for identifying that type of hydrophobic domain. The exact boundaries of a transmembrane domain may vary but most likely by no more than about 5 amino acids at either end of the domain specifically mentioned herein. Accordingly, the APRIL-R ECD may optionally comprise amino acids 8-41 (SEQ ID NO:1).

"APRIL-R variant" means an active APRIL-R as defined below having at least about 80% amino acid sequence identity with the APRIL-R having the deduced amino acid



sequence shown in SEQ ID NO:1 for a full-length native sequence APRIL-R or with a APRIL-R ECD sequence. Such APRIL-R variants include, for instance, APRIL-R polypeptides wherein one or more amino acid residues are added, or deleted, at the end or C-terminus of the sequence of SEQ ID NO:1. Ordinarily, a APRIL-R variant will have at least about 80% or 85% amino acid sequence identity, more preferably at least about 90% amino acid sequence identity, and even more preferably at least about 95% amino acid sequence identity with the amino acid sequence of SEQ ID NO:1.

"Percent (%) amino acid sequence identity" with respect APRIL-R sequences identified herein is defined as the percentage of amino acid residues in a candidate sequence that are identical with the amino acid residues in the APRIL-R sequence, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity. Alignment for purposes of determining percent amino acid sequence identity can be achieved in various ways that are within the skill in the art, for instance, using publically available computer software such as BLAST, ALIGN, or Megalign (DNASTAR) software. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximum alignment over the full length of the sequences being compared.

The term "epitope tagged" when used herein refers to a chimeric polypeptide comprising APRIL-R, or a domain sequence thereof, fused to a "tag polypeptide". The tag polypeptide has enough residues to provide an epitope against which an antibody can be made, or which can be identified by some other agent, yet is short enough such that it does not interfere with activity of the APRIL-R. The tag polypeptide preferably also is fairly unique so that the antibody does not substantially cross-react with other epitopes. Suitable tag polypeptides generally have at least 6 amino acid residues and usually between about 8 to about 50 amino acid residues (preferably, about 10 to about 20 residues).

"Isolated" when used to describe the various polypeptides disclosed herein, means polypeptide that has been identified and separated and/or recovered from a component of its natural environment. Contaminate components of its natural environment are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and



may include enzymes, hormones, and other proteinaceous or non- proteinaceous solutes. In preferred embodiments, the polypeptide will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by us of a spinning cup sequenator, or (2) to homogeneity SDS-PAGE under non-reducing or  
5 reducing conditions using Coomassie blue or preferably, silver stain. Isolated polypeptide includes polypeptide *in situ* within recombinant cells, since at least one component of the APRIL-R's natural environment will not be present. Ordinarily, however, isolated polypeptide will be prepared by at least one purification step.

The term "antibody" is used in the broadest sense and specifically covers single  
10 APRIL-R monoclonal antibodies (including agonist, antagonist, and neutralizing antibodies) and anti- APRIL-R antibody compositions with polypepitopic specificity. The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e. the individual antibodies comprising the population are identical except for possible naturally-occurring mutations  
15 that may be present in minor amounts.

A "purified preparation" or a "substantially pure preparation" of a polypeptide, as used herein, means a polypeptide that has been separated from other proteins, lipids, and nucleic acids with which it naturally occurs. Preferably, the polypeptide is also separated from other substances, e.g., antibodies, matrices, etc., which are used to purify it.

20 The terms, "treating", "treatment" and "therapy" as used herein refers to curative therapy, prophylactic therapy, and preventative therapy.

The terms "peptides", "proteins", and "polypeptides" are used interchangeably herein.

"Biologically active" as used herein, means having an *in vivo* or *in vitro* activity  
25 which may be performed directly or indirectly. Biologically active fragments of APRIL-R may have, for example, 70% amino acid homology with the active site of the receptor, more preferably at least 80%, and most preferably, at least 90% homology. Identity or homology with respect to the receptor is defined herein as the percentage of amino acid residues in the candidate sequence which are identical to the APRIL-R residues in SEQ.

30 ID. NO. 1.



The term "mammal" as used herein refers to any animal classified as a mammal including humans, cows, horses, dogs, mice and cats. In preferred embodiment of the invention, the mammal is a human.

The practice of the present invention will employ, unless otherwise indicated, conventional techniques of cell biology, cell culture, molecular biology, transgenic biology, microbiology, recombinant DNA, and immunology, which are within the skill of the art. Such techniques are described in the literature.

Reference will now be made in detail to the present preferred embodiments of the invention. This invention relates to the use of APRIL-R and APRIL-R related molecules to effect the growth and maturation of B-cells and non-B cells, specifically as they relate to tumor cells. The invention also relates to the use of APRIL-R and APRIL-R related molecules to effect responses of the immune system, as necessitated by immune-related disorders. Additionally, this invention encompasses the treatment of cancer and immune disorders through the use of a APRIL-R, or APRIL-R related gene through gene therapy methods.

The APRIL-R and homologs thereof produced by hosts transformed with the sequences of the invention, as well as native APRIL-R purified by the processes known in the art, or produced from known amino acid sequences, are useful in a variety of methods for anticancer, antitumor and immunoregulatory applications. They are also useful in therapy and methods directed to other diseases.

Another aspect of the invention relates to the use of the polypeptide encoded by the isolated nucleic acid encoding the APRIL-R in "antisense" therapy. As used herein, "antisense" therapy refers to administration or *in situ* generation of oligonucleotides or their derivatives which specifically hybridize under cellular conditions with the cellular mRNA and/or DNA encoding the ligand of interest, so as to inhibit expression of the encoded protein, i.e. by inhibiting transcription and/or translation. The binding may be by conventional base pair complementarity, or, for example, in the case of binding to DNA duplexes, through specific interactions in the major groove of the double helix. In general, "antisense" therapy refers to a range of techniques generally employed in the art, and includes any therapy which relies on specific binding to oligonucleotide sequences.





An antisense construct of the present invention can be delivered, for example, as an expression plasmid, which, when transcribed in the cell, produces RNA which is complementary to at least a portion of the cellular mRNA which encodes Kay-ligand. Alternatively, the antisense construct can be an oligonucleotide probe which is generated  
5 ex vivo. Such oligonucleotide probes are preferably modified oligonucleotides which are resistant to endogenous nucleases, and are therefor stable in vivo. Exemplary nucleic acids molecules for use as antisense oligonucleotides are phosphoramidates, phosphothioate and methylphosphonate analogs of DNA (See, e.g., 5,176,996; 5,264,564; and 5,256,775). Additionally, general approaches to constructing oligomers useful in antisense therapy  
10 have been reviewed, for example, by Van Der Krol et al., (1988) *Biotechniques* 6:958-976; and Stein et al. (1988) *Cancer Res* 48: 2659-2668, specifically incorporated herein by reference.

The APRIL-R of the invention, as discussed above, is a member of the TNF receptor family. The protein, fragments or homologs thereof may have wide therapeutic  
15 and diagnostic applications.

The polypeptides of the invention specifically interact with APRIL, a polypeptide previously described in WO99/12964 incorporated by reference herein. However, the peptides and methods disclosed herein enable the identification of molecules which specifically interact with the APRIL-R or fragments thereof.

20 The claimed invention in certain embodiments includes methods of using peptides derived from APRIL-R which have the ability to bind to APRIL. Fragments of the APRIL-Rs can be produced in several ways, e.g., recombinantly, by PCR, proteolytic digestion or by chemical synthesis. Internal or terminal fragments of a polypeptide can be generated by removing one or more nucleotides from one end or both ends of a nucleic  
25 acid which encodes the polypeptide. Expression of the mutagenized DNA produces polypeptide fragments.

Polypeptide fragments can also be chemically synthesized using techniques known in the art such as conventional Merrifield solid phase f-moc or t-boc chemistry. For example, peptides and DNA sequences of the present invention may be arbitrarily divided  
30 into fragments of desired length with no overlap of the fragment, or divided into



overlapping fragments of a desired length. Methods such as these are described in more detail below.

#### Generation of Soluble Forms of APRIL-R

Soluble forms of the APRIL-R can often signal effectively and hence can be administered as a drug which now mimics the natural membrane form. It is possible that the APRIL-R claimed herein are naturally secreted as soluble cytokines, however, if not, one can reengineer the gene to force secretion. To create a soluble secreted form of APRIL-R, one would remove at the DNA level the N-terminus transmembrane regions, and some portion of the stalk region, and replace them with a type I leader or alternatively a type II leader sequence that will allow efficient proteolytic cleavage in the chosen expression system. A skilled artisan could vary the amount of the stalk region retained in the secretion expression construct to optimize both ligand binding properties and secretion efficiency. For example, the constructs containing all possible stalk lengths, i.e. N-terminal truncations, could be prepared such that proteins starting at amino acids 1 to 52 would result. The optimal length stalk sequence would result from this type of analysis.

#### Generation of Antibodies Reactive with the APRIL-R

The invention also includes antibodies specifically reactive with the claimed APRIL-R or its co-receptors. Anti-protein/anti-peptide antisera or monoclonal antibodies can be made by standard protocols (See, for example, *Antibodies: A Laboratory Manual* ed. by Harlow and Lane (Cold Spring Harbor Press: 1988)). A mammal such as a mouse, a hamster or rabbit can be immunized with an immunogenic form of the peptide. Techniques for conferring immunogenicity on a protein or peptide include conjugation to carriers, or other techniques, well known in the art.

An immunogenic portion of APRIL-R or its co-receptors can be administered in the presence of an adjuvant. The progress of immunization can be monitored by detection of antibody titers in plasma or serum. Standard ELISA or other immunoassays can be used with the immunogen as antigen to assess the levels of antibodies.

In a preferred embodiment, the subject antibodies are immunospecific for antigenic determinants of APRIL-R or its co-receptors, e.g. antigenic determinants of a polypeptide of SEQ. ID. NO:1, or a closely related human or non-human mammalian homolog (e.g. 70, 80 or 90 percent homologous, more preferably at least 95 percent homologous). In yet a



further preferred embodiment of the present invention, the anti-APRIL-R or anti-APRIL-co-receptor antibodies do not substantially cross react (i.e. react specifically) with a protein which is e.g., less than 80 percent homologous to SEQ. ID. NO:1; preferably less than 90 percent homologous with SEQ. ID. NO: 1; and, most preferably less than 95 percent homologous with SEQ. ID. NO:1. By "not substantially cross react", it is meant that the antibody has a binding affinity for a non-homologous protein which is less than 10 percent, more preferably less than 5 percent, and even more preferably less than 1 percent, of the binding affinity for a protein of SEQ. ID. NO. 1.

The term antibody as used herein is intended to include fragments thereof which are also specifically reactive with APRIL-R, or its receptors. Antibodies can be fragmented using conventional techniques and the fragments screened for utility in the same manner as described above for whole antibodies. For example, F(ab')<sub>2</sub> fragments can be generated by treating antibody with pepsin. The resulting F(ab')<sub>2</sub> fragment can be treated to reduce disulfide bridges to produce Fab' fragments. The antibodies of the present invention are further intended to include biospecific and chimeric molecules having anti-APRIL-R or anti-APRIL-co-receptor activity. Thus, both monoclonal and polyclonal antibodies (Ab) directed against APRIL-R, and their co-receptors, and antibody fragments such as Fab' and F(ab')<sub>2</sub>, can be used to block the action of the APRIL-R and its respective co-receptors.

Various forms of antibodies can also be made using standard recombinant DNA techniques. (Winter and Milstein, Nature 349: 293-299 (1991) specifically incorporated by reference herein.) For example, chimeric antibodies can be constructed in which the antigen binding domain from an animal antibody is linked to a human constant domain (e.g. Cabilly et al., U.S. 4,816,567, incorporated herein by reference). Chimeric antibodies may reduce the observed immunogenic responses elicited by animal antibodies when used in human clinical treatments.

In addition, recombinant "humanized antibodies" which recognize APRIL-R or its co-receptors can be synthesized. Humanized antibodies are chimeras comprising mostly human IgG sequences into which the regions responsible for specific antigen-binding have been inserted. Animals are immunized with the desired antigen, the corresponding antibodies are isolated, and the portion of the variable region sequences responsible for



specific antigen binding are removed. The animal-derived antigen binding regions are then cloned into the appropriate position of human antibody genes in which the antigen binding regions have been deleted. Humanized antibodies minimize the use of heterologous (i.e. inter species) sequences in human antibodies, and thus are less likely to elicit immune responses in the treated subject.

Construction of different classes of recombinant antibodies can also be accomplished by making chimeric or humanized antibodies comprising variable domains and human constant domains (CH1, CH2, CH3) isolated from different classes of immunoglobulins. For example, antibodies with increased antigen binding site valencies can be recombinantly produced by cloning the antigen binding site into vectors carrying the human  $\gamma$  chain constant regions. (Arulanandam et al., J. Exp. Med., 177: 1439-1450 (1993), incorporated herein by reference.)

In addition, standard recombinant DNA techniques can be used to alter the binding affinities of recombinant antibodies with their antigens by altering amino acid residues in the vicinity of the antigen binding sites. The antigen binding affinity of a humanized antibody can be increased by mutagenesis based on molecular modeling. (Queen et al., Proc. Natl. Acad. Sci. 86: 10029-33 (1989) incorporated herein by reference.

#### Generation of Analogs: Production of Altered DNA and Peptide Sequences

Analogs of the APRIL-R can differ from the naturally occurring APRIL-R in amino acid sequence, or in ways that do not involve sequence, or both. Non-sequence modifications include in vivo or in vitro chemical derivatization of the APRIL-R. Non-sequence modifications include, but are not limited to, changes in acetylation, methylation, phosphorylation, carboxylation or glycosylation.

Preferred analogs include APRIL-R biologically active fragments thereof, whose sequences differ from the sequence given in SEQ. ID NO. 1, by one or more conservative amino acid substitutions, or by one or more non-conservative amino acid substitutions, deletions or insertions which do not abolish the activity of APRIL-ligand. Conservative substitutions typically include the substitution of one amino acid for another with similar characteristics, e.g. substitutions within the following groups: valine, glycine; glycine, alanine; valine, isoleucine, leucine; aspartic acid, glutamic acid; asparagine, glutamine; serine, threonine; lysine, arginine; and, phenylalanine, tyrosine.





### Uses

The full length APRIL-R gene (SEQ ID NO 2) or portions thereof may be used as hybridization probes for a cDNA library to isolate, for instance, still other genes which have a desired sequence identity to the APRIL-R sequence disclosed in SEQ ID NO. 2.

5 Nucleotide sequences encoding APRIL-R can also be used to construct hybridization probes for mapping the gene which encodes the APRIL-R and for the genetic analysis of individuals with genetic disorders. Screening assays can be designed to find lead compounds that mimic the biological activity of a APRIL-R. Such screening assays will include assays amenable to high-throughput screening of chemical libraries, making them  
10 particularly suitable for identifying small molecule drug candidates. Small molecules contemplated include synthetic organic or inorganic compounds. Nucleic acids which encode APRIL-R or its modified forms can also be used to generate either transgenic animals or "knock out" animals which in turn are useful in the development and screening of therapeutically useful reagents.

15 As described herein, in one embodiment of the invention, there are provided methods of inhibiting B-cell and non-B cell growth, dendritic cell-induced B-cell growth and maturation or immunoglobulin production in an animal using APRIL-R polypeptide.

In another embodiment, the invention provides methods of using APRIL-R in the treatment of autoimmune diseases, hypertension, cardiovascular disorders, renal disorders,  
20 B-cell lympho-proliferate disorders, immunosuppressive diseases, organ transplantation, inflammation, and HIV. Also included are methods of using agents for treating, suppressing or altering an immune response involving a signaling pathway between APRIL-R and its ligand.

In one embodiment, the invention provides pharmaceutical compositions  
25 comprising a APRIL-R polypeptide and a pharmaceutically acceptable excipient. Suitable carriers for a APRIL-R polypeptide, for instance, and their formulations, are described in Remington' Pharmaceutical Sciences, 16<sup>th</sup> ed., 1980, Mack Publishing Co., edited by Oslo et al. Typically an appropriate amount of a pharmaceutically acceptable salt is used in the formulation to render the formulation isotonic. Examples of the carrier include buffers  
30 such as saline, Ringer's solution and dextrose solution. The pH of the solution is preferably from about 5 to about 8, and more preferably from about 7.4 to about 7.8.



Further carriers include sustained release preparations such as semipermeable matrices of solid hydrophobic polymers, which matrices are in the form of shaped articles, e.g. liposomes, films or microparticles. It will be apparent to those of skill in the art that certain carriers may be more preferable depending upon for instance the route of administration and concentration of the a APRIL-R polypeptide being administered.

Administration may be accomplished by injection (eg intravenous, intraperitoneal, subcutaneous, intramuscular) or by other methods such as infusion that ensure delivery to the bloodstream in an effective form.

Practice of the present invention will employ, unless indicated otherwise, conventional techniques of cell biology, cell culture, molecular biology, microbiology, recombinant DNA, protein chemistry, and immunology, which are within the skill of the art. Such techniques are described in the literature. See, for example, *Molecular Cloning: A Laboratory Manual*, 2nd edition. (Sambrook, Fritsch and Maniatis, eds.), Cold Spring Harbor Laboratory Press, 1989; *DNA Cloning*, Volumes I and II (D.N. Glover, ed), 1985; *Oligonucleotide Synthesis*, (M.J. Gait, ed.), 1984; U.S. Patent No. 4,683,195 (Mullis et al.); *Nucleic Acid Hybridization* (B.D. Hames and S.J. Higgins, eds.), 1984; *Transcription and Translation* (B.D. Hames and S.J. Higgins, eds.), 1984; *Culture of Animal Cells* (R.I. Freshney, ed). Alan R. Liss, Inc., 1987; *Immobilized Cells and Enzymes*, IRL Press, 1986; *A Practical Guide to Molecular Cloning* (B. Perbal), 1984; *Methods in Enzymology*, Volumes 154 and 155 (Wu et al., eds), Academic Press, New York; *Gene Transfer Vectors for Mammalian Cells* (J.H. Miller and M.P. Calos, eds.), 1987, Cold Spring Harbor Laboratory; *Immunochemical Methods in Cell and Molecular Biology* (Mayer and Walker, eds.), Academic Press, London, 1987; *Handbook of Experiment Immunology*, Volumes I-IV (D.M. Weir and C.C. Blackwell, eds.), 1986; *Manipulating the Mouse Embryo*, Cold Spring Harbor Laboratory Press, 1986.

The following Examples are provided to illustrate the present invention, and should not be construed as limiting thereof.

#### EXAMPLES:

30

The following methods were used in the Examples disclosed hereinafter.



## Methods:

Cloning and expression of myc-tagged murine APRIL (CCM776) in *Pichia pastoris*.

- 5 The expression vector pCCM213.10 was constructed by taking PDR004 (H98 muAPRIL with superFAS-ligand stalk attached to N terminus along with FLAG epitope tag) and excising out the mu APRIL coding sequence from Sac I to NotI. Synthetic oligonucleotides LTB-559 and 560 form a Xho-I-SacI linker which contain an alpha mating factor leader sequence, myc epitope tag, as well as the KEL motif from FAS
- 10 ligand. Both the muAPRIL fragment and linker were ligated into the Xho-I-NotI sites of pccm211, a *Pichia pastoris* expression plasmid.
- PCCM213.10 was linearized with StuI, electroporated into GS115 strain (his4-) and plated into minimal media containing dextrose. HIS4 transformants were analyzed for protein expression by inoculating a single representative colony in rich media (BMGY: Buffered
- 15 glycerol complex medium) and allowing it to grow to density for 48 hours at 30C. Cultures were spun, and cell pellets were resuspended (1:5) in a rich induction media containing 1.5% methanol (BMMY:Buffered methanol complex media). After two days of induction at 30C, supernatants were run out on SDS-PAGE and assessed for the presence of muAPRIL. Coomassie staining and Western blot (with the anti-myc mAb 9E10)
- 20 showed that one strain, CCM776, produced adequate amounts of the glycosylated form myc-tagged-H98 muAPRIL protein.

## Myc-mAPRIL purification

- Myc-mApril, a protein of 149 amino acids was expressed in *pichia*. This protein
- 25 has an isoelectric point of 7.45. 175 ml of *pichia* supernatant was dialyzed and buffer exchanged to to 10mM Tris pH 6.8 overnight and then passed through a 20 ml SP column. The column was washed extensively with 10mM Tris-HCl, pH 6.8, and eluted with 250mM NaCl in PBS. A second step purification was achieved using a gel filtration column (S300). Fractions containing myc-April from 20 ml SP column were concentrated by
- 30 centrifugation to a volume of 7 ml. After gel filtration, we recovered 8 mg of myc-APRIL as detected by OD and coomassie gel. We also performed Western blot analysis using



mouse monoclonal 9E10 antibody (anti-myc) showing that the myc tag is intact after the purification steps. N terminal sequence verified that the purified protein corresponds to myc-mApril.

5 **FLAG-human April purification.**

Plasmid ps429 (subsequently named p1448) was used to transiently transfect 293 T cells using lipofectamine reagent (Gibco-Brl) and serum free media. The plasmid, constructed in the mammalian expression vector PCR3 (Invitrogen) encodes the receptor-binding domain of human APRIL, with an N-terminal protein into the cell culture media.

10 FLAG-APRIL protein was purified from serum free media using an anti-FLAG mAb M@ column and excess purified FLAG peptide, following the manufacturers' instructions (Kodak).

**HBMCA-Fc purification.**

15 HBMCA-Fc was transiently transfected into 293 cells. Conditioned media from 293 cells over-expressing hBCM-Fc was loaded into a protein A column. Protein was eluted using 25 mM phosphate 100mM NaCl pH 2.8 followed by neutralization with 1/20 volume of 0.5 M NaPO4 pH 8.6. Selected fractions based in OD 280 were subject to reducing and non-reducing SDS-PAGE gels and western blots to identify the purified protein. 3 mg of  
20 protein were recovered from 500 ml of conditioned media.

**Myc-mAPRIL binds to various cell lines in FACS analysis.**

450 ng/ml of purified myc-mAPRIL was bound to cell lines in 100ul PBS/2%FBS + Fc blocking reagents (FcBlock @ 20ug/ml (Pharmlngen) and purified human IgG @ 10  
25 ug/ml (Sandoz) on ice for 1 hour. Positive binding was revealed using specific rabbit anti-murine APRIL antisera (1:500) and donkey anti-rabbit IgG-FITC (Jackson). Cell lines A20, Raji, NIH3T3, and HT29 were maintained in media as suggested by the supplier (ATCC Bethesda, MD). BJAB cells were cultured in HEPES-buffered RPMI supplemented with 10% FBS and L-glutamine. In competition assays 450ng/ml myc-  
30 murine APRIL was added with 1 ug/ml of competitor protein.





### Example 1: Detection of APRIL binding to APRIL-R using a Plate Assay

In this example, we tested whether BCMA associates with April.

In order to test whether BCMA associates with April we performed a co-immunoprecipitation experiment. Both soluble proteins hBCMA-Fc and myc-mApril were used in this experiment.

hBCMA-Fc and LTbR-Fc were added with different TNF ligands: myc-mApril; myc-CD40L and myc-RANKL into media containing 10% FBS for ½ hour at room temperature. Fc proteins were bound to protein A beads for 1-2 hours, washed three times with 1 ml of PBS, analyzed by immunoblotting with mouse monoclonal 9E10 (anti-myc) antibody and developed using enhanced chemiluminescence.

We could detect myc-APRIL in hBCMA-Fc immunoprecipitates indicating that BCMA interacts with April in a specific way since other TNF ligands, myc-CD40L and myc-RANKL did not have the ability to bind to BCMA. Myc-April does not associate with LTbR-Fc.

The same membrane was stripped and reblotted with anti-hIG-HRP to show that the same amount of LTbR-Fc with BCMA-Fc were used in the immunoprecipitates.

### Example 2:

This example describes that hBCMA-Fc interacts with FLAG-hAPRIL.

ELISA analysis: Coated plates with receptor-Fc fusion proteins (hBCMA-Fc-739 or hTNFR2-Fc-492) at 1 ug/ml in carbonate pH 9.6, overnight, 4C. Blocked for 2 hours at room temperature using PBS/5% non fat dry milk/0.5% Tween-20. 2x serial dilution of ligands were made in 100 ul of blocking buffer (TNFa-197 from 1000ng/ml, muBAFF-657 from 1000ng/ml, hApril-507 from 2000 ng/ml (inactive), hApril-429 from 5x concentrated media). After incubation with ligands the plate was washed in PBS) .5% Tween-20 and probed with 0.5ug/ml anti-FLAG mAb M2 in dilution buffer. The antibody was then detected using anti-mouse-PO 1/2000 with enzymatic development (OPD).

Immunoprecipitation experiments: 293T cells were transfected with indicated expression plasmid (Rec-Fc or flag ligand) in 9 cm plate. Transfected cells were left for 5d in 8ml Optimem media (Gibco-BRL). Immunoprecipitation were performed by mixing



200 ul of each receptor-conditioned media with 200 ul of each ligand-conditioned media + 400 ul PBS + 10 ul ProtG-Sepharose. These were rotated 1h on a wheel, washed 4x with 1ml PBS, then boiled in 50 ul sample buffer (+DTT). Loaded 20 ul of each immunoprecipitation per lane. Reveal blot with 1ug/ml anti-FLAG M2 mAb (Sigma, St Louis MO) and anti-mouse PO (1/2000). Reprobe blot with anti-human-PO was also checked: Take 100 ul conditioned media and precipitate with MeOH/CHCl<sub>3</sub>/lysozyme. Boil in 50ul sample buffer (+DTT). Load 20 ul. Reveal blot with anti-FLAG mAb M2 (1ug/ml) and anti-mouse-PO (1/2000).

10 **Example 3:**

This example describes the binding of myc-mAPRIL; hKayL-440 (hBAFF); and Flag-mBAFF to hBCMA-Ig, hLT-R-Ig, or hp80 TNFR-Ig. All experiments were performed at 25C with a 10  $\mu$ /ml minute flow rate.

Each experiment was performed using HBS buffer (10mM HEPES, 150 mM NaCl, 0.005% P20 surfactant, at pH 7.4). The same solution was used both as running buffer and as sample diluent.

The CM5 chip (BIAcore, Inc.) surface was first activated with N-hydroxysuccinimide/N-ethyl-N'-(3-diethylaminopropyl)-carbodiimide hydrochloride (BIAcore). Twenty  $\mu$ l of hBCMA-Ig; fifteen  $\mu$ l of hLT-R05-Ig and 10  $\mu$ l of hp80-TNFR, diluted to 30g/ml in 10 mM acetic acid were then blocked with once with 30  $\mu$ l and again with 15  $\mu$ l of ethanolamine-HCL (pH 8.5). This resulted in a surface density of 1600-3700 resonance units (RU). The chip was regenerated with 20  $\mu$ l of 1mM formic acid. These rejections were repeated five times to establish a reproducible and stable baseline.

For the experiment, 100  $\mu$ l of myc-mApril, hKayL-440, and FLAG-mBAFF each was diluted to 30  $\mu$ g/ml in diluent buffers and was injected over the surface of the chip. Immediately after each injection, the chip was washed with 500  $\mu$ l of the diluent buffer. The surface was regenerated between experiments by injecting 20  $\mu$ l of 1 mM formic acid; followed with another 15  $\mu$ l injection formic acid. After regeneration, the chip was equilibrated with the dilution buffer.

30



#### Example 4: Generation of Soluble Receptor Forms:

To form an receptor inhibitor for use in man, one requires the human receptor cDNA sequence of the extracellular domain. If the mouse form is known, human cDNA libraries can be easily screened using the mouse cDNA sequence and such manipulations are routinely carried out in this area. With a human cDNA sequence, one can design oligonucleotide primers to PCR amplify the extracellular domain of the receptor in the absence of the transmembrane and intracellular domains. Typically, one includes most of the amino acids between the last disulfide linked "TNF domain" and the transmembrane domain. One could vary the amount of "stalk" region included to optimize the potency of the resultant soluble receptor. This amplified piece would be engineered to include suitable restriction sites to allow cloning into various C-terminal Ig fusion chimera vectors. Alternatively, one could insert a stop signal at the 3' end and make a soluble form of the receptor without resorting to the use of a Ig fusion chimera approach. The resultant vectors can be expressed in most systems used in biotechnology including yeast, insect cells, bacteria and mammalian cells and examples exist for all types of expression. Various human Fc domains can be attached to optimize or eliminate FcR and complement interactions as desired. Alternatively, mutated forms of these Fc domains can be used to selectively remove FcR or complement interactions or the attachment of N-linked sugars to the Fc domain which has certain advantages.

#### Example 5: Generation of Agonistic or Antagonistic Antibodies:

The above described soluble receptor forms can be used to immunize mice and to make monoclonal antibodies by conventional methods. The resultant mAbs that are identified by ELISA methods can be further screened for agonist activity either as soluble antibodies or immobilized on plastic in various in vitro cellular assays. Often the death of the HT29 cell line is a convenient system that is sensitive to signaling through many TNF receptors. If this line does not possess the receptor of interest, that full length receptor can be stably transfected into the HT29 line to now allow the cytotoxicity assay to work.



Alternatively, such cells can be used in the Cytosensor apparatus to assess whether activation of the receptor can elicit a pH change that is indicative of a signaling event. TNF family receptors signal well in such a format and this method does not require one to know the actual biological events triggered by the receptor. The agonistic mAbs would be "humanized" for clinical use. This procedure can also be used to define antagonistic mAbs. Such mAbs would be defined by the lack of agonist activity and the ability to inhibit receptor-ligand interactions as monitored by ELISA, classical binding or BIAcore techniques. Lastly, the induction of chemokine secretion by various cells in response to an agonist antibody can form a screening assay.

10

#### Example 6: Screening for Inhibitors of the Receptor-Ligand Interaction:

Using the receptor-Ig fusion protein, one can screen either combinatorial libraries for molecules that can bind the receptor directly. These molecules can then be tested in an ELISA formatted assay using the receptor-Ig fusion protein and a soluble form of the ligand for the ability to inhibit the receptor-ligand interaction. This ELISA can be used directly to screen various natural product libraries etc. for inhibitory compounds. The receptor can be transfected into a cell line such as the HT29 line to form a biological assay (in this case cytotoxicity) that can then form the screening assay.

20 It will be apparent to those skilled in the art that various modifications and variations can be made in the polypeptides, compositions and methods of the invention without departing from the spirit or scope of the invention. Thus, it is intended that the present invention cover the modifications and variations of this invention provided that they come within the scope of the appended claims and their equivalents.

25





What is claimed is:

1. A method of inhibiting B-cell growth in an animal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of:
  - (a) a anti-APRIL-R molecule or an active fragment thereof;
  - (b) a recombinant, inoperative APRIL-R molecule or an active fragment thereof;
  - (c) an antibody specific for APRIL-R or an active fragment thereof; and
  - (d) an antibody specific for APRIL-R or an epitope thereof.
2. The method according to claim 1, wherein the anti-APRIL-R is soluble.
3. The method according to claim 2, wherein the soluble anti-APRIL-R is a recombinant anti-APRIL-R.
4. The method according to claim 1, wherein the anti-APRIL antibody is a monoclonal antibody.
5. The method according to claim 1, wherein the animal is of mammalian origin.
6. The method according to claim 5, wherein the mammal is human.
7. A pharmaceutical composition comprising a therapeutically effective amount of an isolated APRIL-R polypeptide or a fragment thereof and a pharmaceutically acceptable carrier.
8. The isolated APRIL-R polypeptide of claim 7 wherein the APRIL-R polypeptide is selected from the group consisting of:
  - a) an isolated native sequence APRIL-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO:1 or a fragment thereof;
  - b) an isolated APRIL-R polypeptide having at least 80% amino acid sequence identity with native sequence APRIL-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO: 1 or a fragment thereof;
  - c) an isolated APRIL-R polypeptide having at least 90% amino acid sequence identity with native sequence APRIL-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO: 1 or a fragment thereof;
  - d) an isolated sequence APRIL-R polypeptide comprising amino acid residues 1 to 52 of SEQ ID NO. 1 or a fragment thereof; and



e) an isolated sequence APRIL-R polypeptide comprising amino acid residues 8 to 41 of SEQ ID NO. 1 or a fragment thereof.

9. A chimeric molecule comprising:

a) a APRIL-R polypeptide selected from the group consisting of:

i) an isolated native sequence APRIL-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO:1 or a fragment thereof;

ii) an isolated APRIL-R polypeptide having at least 80% amino acid sequence identity with native sequence APRIL-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO: 1 or a fragment thereof;

ii) an isolated APRIL-R polypeptide having at least 90% amino acid sequence identity with native sequence APRIL-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO: 1 or a fragment thereof;

iii) an isolated sequence APRIL-R polypeptide comprising amino acid residues 1 to 52 of SEQ ID NO. 1 or a fragment thereof; and

iv) an isolated sequence APRIL-R polypeptide comprising amino acid residues 8 to 41 of SEQ ID NO. 1 or a fragment thereof,

b) fused to a heterologous amino acid sequence.

10. The chimeric molecule of claim 9 wherein the heterologous amino acid sequence is an immunoglobulin sequence.

11. The chimeric molecule of claim 10 wherein the immunoglobulin sequence is an IgG Fc domain.

12. An antibody which binds to a APRIL-R polypeptide selected from the group consisting of:

i) an isolated native sequence APRIL-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO:1 or a fragment thereof;

ii) an isolated APRIL-R polypeptide having at least 80% amino acid sequence identity with native sequence APRIL-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO: 1 or a fragment thereof;

ii) an isolated APRIL-R polypeptide having at least 90% amino acid sequence identity with native sequence APRIL-R polypeptide comprising amino acid residues 1 to 184 of SEQ ID NO: 1 or a fragment thereof;



- iii) an isolated sequence APRIL-R polypeptide comprising amino acid residues 1 to 52 of SEQ ID NO. 1 or a fragment thereof; and
- iv) an isolated sequence APRIL-R polypeptide comprising amino acid residues 8 to 41 of SEQ ID NO. 1 or a fragment thereof.

- 5 13. The antibody of claim 12 wherein the antibody is a monoclonal antibody.
- 14. The antibody of claim 12 which comprises a chimeric antibody.
- 15. The antibody of claim 12 which comprises a human antibody.
- 16. A method of therapeutically treating a mammal for a condition associated with undesired cell proliferation, said method comprising administering to said mammal a therapeutically effective amount of a composition comprising an APRIL-R antagonist with a pharmaceutically acceptable recipient.
- 10 17. The method of claim 16, wherein the APRIL-R antagonist comprises a polypeptide that antagonizes the interaction between APRIL and its cognate receptor or receptors.
- 15 18. The method of claim 17, wherein the APRIL-R antagonist comprises an anti-APRIL-R antibody homolog.
- 20 19. The method of claim 18, wherein the anti-APRIL-R antibody homolog is an anti-BCMA antibody.
- 20. The method of claim 17, wherein the APRIL-R antagonist comprises an anti-APRIL-R homolog.
- 25 21. The method of claim 20, wherein the anti-APRIL-R homolog is BCMA.
- 22. A method of therapeutically treating a mammal for a condition associated with undesired cell proliferation, said method comprising administering to said mammal a therapeutically effective amount of two or more antagonists, wherein at least two of the antagonists include a first APRIL-R antagonist that antagonizes the interaction between APRIL and BCMA, and a second APRIL-R antagonist that antagonizes an interaction between APRIL and another cognate APRIL receptor or receptors that are not BCMA.
- 30 23. A method of inhibiting non-B-cell growth in a mammal comprising the step of administering a therapeutically effective amount of a composition selected from the group consisting of:
  - (e) a anti-APRIL-R molecule or an active fragment thereof;
  - (f) a recombinant, inoperative APRIL-R molecule or an active fragment thereof;
  - (g) an antibody specific for APRIL-R or an active fragment thereof; and
  - (h) an antibody specific for APRIL-R or an epitope thereof.
- 40



24. The method according to claim 23, wherein the anti-APRIL-R is soluble.
- 5 25. The method according to claim 24, wherein the soluble anti-APRIL-R is a recombinant anti-APRIL-R.
26. The method according to claim 23, wherein the anti-APRIL antibody is a monoclonal antibody.
- 10 27. The method according to claim 23, wherein the animal is of mammalian origin.
28. The method according to claim 27, wherein the mammal is human.
- 15 29. A method of inhibiting non-B-cell growth in a mammal comprising administering to said mammal a therapeutically effective amount of two or more antagonists, wherein at least two of the antagonists include a first APRIL-R antagonist that antagonizes the interaction between APRIL and BCMA, and a second APRIL-R antagonist that antagonizes an interaction between APRIL and another cognate APRIL receptor or receptors that are not BCMA.
- 20 30. A method of inhibiting B-cell growth in a mammal comprising administering to said mammal a therapeutically effective amount of two or more antagonists, wherein at least two of the antagonists include a first APRIL-R antagonist that antagonizes the interaction between APRIL and BCMA, and a second APRIL-R antagonist that antagonizes an interaction between APRIL and another cognate
- 25 APRIL receptor or receptors that are not BCMA





# ABSTRACT

A novel receptor in the TNF family is provided: APRIL-R. Chimeric molecules and antibodies to APRIL-R and methods of use thereof are also provided.

5

APRIL-R



Figure 1. Map of myc-murine APRIL construct pCCM213.10 for expression in Yeast

CCAAAG AGG AGA TTT CCT TCA ATT TTT ACT GCA GTT TTA TTC GCA GCA TCC TCC GCA TTA GCT GCT CCA GTC AAC ACT ACA  
GGTTTC TAC TCT TAA AGG AGT TAA AAA TGA CGT CAA AAT AAG CGT AGG AGG AGG AGT AAT CGA CGA GGT CAG TTG TGA TGT  
M R F P S I F T A V L F A A S S A L A A P V N T T  
ACA GAA GAT GAA ACG GCA CAA AAT CCG GCT GAA GCT GTC ATC GGT TAC TCA GAT TTA GAA GGG GAT TTC GAT GTT GCT GTT  
TGT CTT CTA CTT TGC CGT GTT TAA GGC CGA CTT CGA CAG TAG CCA ATG AGT CTA AAT CTT CCC CTA AAG CTA CAA CGA CAA V  
T E D E T A Q I P A E A V I G Y S D L E G D F D V A V  
TTG CCA TTT TCC AAC AGC ACA AAT AAC GGG TTA TTG TTT ATA AAT ACT ACT ATT GCC AGC ATT GCT GCT AAA GAA GAA GGG  
AAC GGT AAA AGG TTG TCG TGT TTA TTG CCC AAT AAC AAA TAT TTA TGA TGA TAA CGG TCG TAA CGA TTT CTT CTT CCC  
L P F S N S T N G L L F I N T T I A S I A A K E E G  
GTA TCT CTC GAG AAA AGA GAA CAA AAA CTC ATT TCT GAG GAA GAT CTG AAT AAA GAG CTC CAC TCA GTC GTC CAT CTT GTT  
CAT AGA GAG CTC TTT TCT CTT GTT TTT GAG TAA AGA CTC CTT CTA GAC TTA TTT CTC GAG GTG AGT CAG GAC GAA GAA CAA

myc-tag      >>>murine APRIL

V S L E K R E Q K L I S E E D L N K E L H S V L L H L V  
CCA GTT AAC ATT ACC TCC AAG GAC TCT GAC GTG ACA GAG GTG ATG TGG CAA CCA GTA CTT AAG CGT GGG AGA GGC CTG GAG  
GGT CAA TTG TAA TGG AGG TTC CTG AGA CTG CAG TGT CTC CAC TAC ACC GGT GGT CAA TCC GCA CCC TCT CCG GAC CTC  
P V N I T S K D S D V T E V M W Q P V L R R G R G L E  
GCC CAG GGA GAC ATT GTA CGA GTC TGG GAC ACT GGA ATT TAT CTG CTC TAT AGT CAG GTC CTG TTT CAT GAT GTG ACT TTC  
CGG GTC CCT CTG TAA CAT GCT CAG ACC CTG TGA CCT TAA ATA GAC GAG ATA TCA GTC CAG GAC AAA GTA CTA CAC TGA AAG  
A Q G D I V R V W D T G I Y L L Y S Q V L F H D V T F  
ACA ATG GGT CAG GTG GTA TCT CCG GAA GGA CAA GGG AGA AGA GAA ACT CTA TTC CGA TGT ATC AGA AGT ATG CCT TCT GAT  
TGT TAC CCA CCA CAC CAT AAG AGC CCA CTT CCG TCT TCT TGA GAT AAG GCT ACA TAG TCT TCA TAG GGA AGA CTA D  
T M G Q V V S R E G Q G R R E T L F R C I R S M P S D  
CCT GAC CGT GCC TAC AAT AGC TGC TAC AGT GCA GGT GTC TTT CAT TTA CAT GAA GGG GAT ATT ATC ACT GTC AAA ATT CCA  
GGA CTG GCA CGG ATG TTA TCG ACG ATG TCA GGT CCA CAG AAA GTA AAT GTA GAT GGT CCC CTA TAA TAG TGA CAG TTT TAA GGT  
P D R A Y N S C Y S A G V F H L H Q G D I I T V K I P  
CGG GCA AAC GCA AAA CTT AGC CTT TCT CCG CAT GGA ACA TTC CTG GGG TTT GTC AAA CTA TGA GCGCGCGGATTAATTCGCTTA  
GCC GGT TTG GGT TTA TCG GAA AGA GGC GGT TGT AAG GAC CCC AAA CAC TTT ACT CGCGCGCGCTTAATTAAGCGAAT  
R A N A K L S L S P H G T F L G F V K L



construct nsA29 for expression in yeast.

Figure 2. Nucleotide sequence of the DNA fragment containing the *lacZ* gene, as determined by the Maxam-Gilbert method. The sequence is presented in the 5' to 3' direction. The *lacZ* gene is indicated by the arrow. The sequence is presented in the 5' to 3' direction. The *lacZ* gene is indicated by the arrow.



1 ATG GAG ACA GAC ACA CTC CTG TTA TGG GTG CTG CTG CTC TGG GTT CCA GGT TCC ACT GGT  
 11 M E T D T L L L W V L L L W V P G S T G  
 501 3 61 GAC GTC ACG ATG TTG CAG ATG GCT GGG CAG TGC TCC CAA AAT GAA TAT TTT GAC AGT TTG  
 11 M L Q M A G Q C S Q N E Y F D S L  
 21 D V T M L Q M A G Q C S Q N E Y F D S L  
 11 TTG CAT GCT TGC ATA OCT TGT CAA CTT CGA TGT TCT TCT AAT ACT OCT CCT CTA ACA TGT  
 18 L H A C I P C Q L R C S S N T P P L T C  
 41 L H A C I P C Q L R C S S N T P P L T C  
 181 CAG CGT TAT TGT AAT GCA AGT GTG ACC AAT TCA GTG AAA GGA GTC GAC AAA ACT CAC ACA  
 38 Q R Y C N A S V T N S V K G V D K T H T  
 61 Q R Y C N A S V T N S V K G V D K T H T  
 241 TGC CCA CCG TGC CCA GCA CCT GAA CTC CTG GGG GGA CCG TCA GTC TTC CTC TTC CCC CCA  
 81 C P P C P A P E L L G G P S V F L F P P  
 301 AAA CCC AAG GAC ACC CTC ATG ATC TCC CCG ACC OCT GAG GTC ACA TGC GTG GTG GTG GAC  
 101 K P K D T L M I S R T P E V T C V V V D  
 361 GTG AGC CAC GAA GAC OCT GAG GTC AAG TTC AAC TGG TAC GTG GAC GGC GTG GAG GTG CAT  
 121 V S H E D P E V K F N W Y V D G V E V H  
 421 AAT GCC AAG ACA AAG CCG CCG GAG GAG CAG TAC AAC AGC ACG TAC CGT GTG GTC AGC GTC  
 141 N A K T K P R E E Q Y N S T Y R V V S V  
 11 CTC ACC GTC CTG CAC CAG GAC TGG CTG AAT GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC  
 11 L T V L H Q D W L N G K E Y K C K V S N  
 1 AAA GCC CTC CCA GCC CCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAA  
 1 K A L P A P I E K T I S K A K G Q P R E  
 1 CCA CAG GTG TAC ACC CTG CCC CCA TCC CCG GAT GAG CTG ACC AAG AAC CAG GTC AGC CTG  
 1 P Q V Y T L P P S R D E L T K N Q V S L  
 1 ACC TGC CTG GTC AAA GGC TTC TAT CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG  
 1 T C L V K G F Y P S D I A V E W E S N G  
 1 CAG CCG GAG AAC AAC TAC AAG ACC ACG OCT CCC GTG TTG GAC TCC GAC GGC TCC TTC TTC  
 1 Q P E N N Y K T T P P V L D S D G S F F  
 1 CTC TAC AGC AAG CTC ACC GTG GAC AAG AGC AGG TGG CAG CAG GGG AAC GTC TTC TCA TGC  
 1 L Y S K L T V D K S R W Q Q G N V F S C  
 1 TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACG CAG AAG AGC CTC TCC CTG TCT CCC  
 281 S V M H E A L H N H Y T Q K S L S L S P  
 901 GGG AAA TGA  
 301 G K

Figure 3

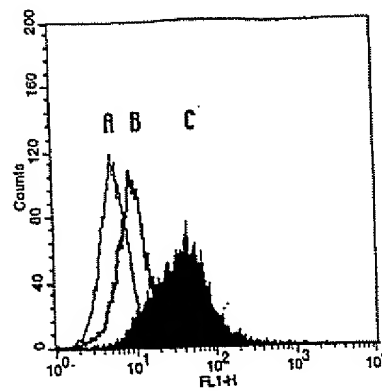




Figure 4

A

A20 cells

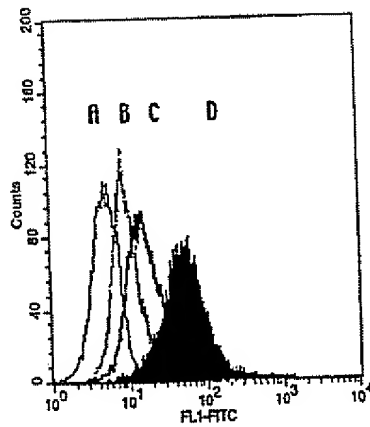


A: Unstained cells  
B: Rabbit sera 1532 control  
C: 450ng APRIL + R1532

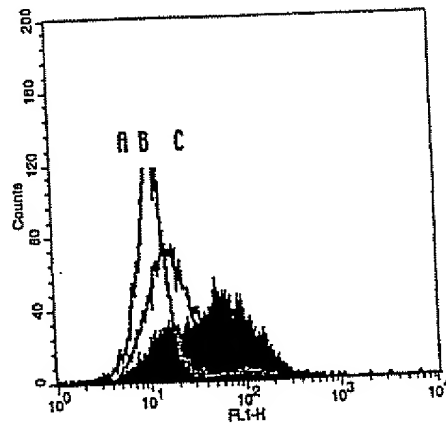
A20 cells

C

A20s



A: unstained cells  
B: Rabbit sera 1532 control  
C: 1 ug Rank-L + R1532  
D: 450ng mAPRIL + 1532



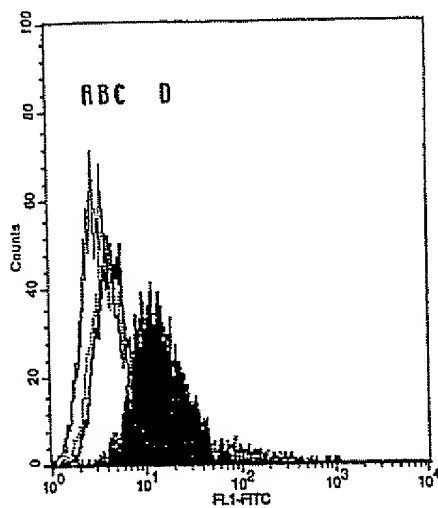
A: 450ngs APRIL + irrelevant rabbit sera  
B: Rabbit sera 1532 control  
C: 450ngs APRIL + R1532



Figure 5

A

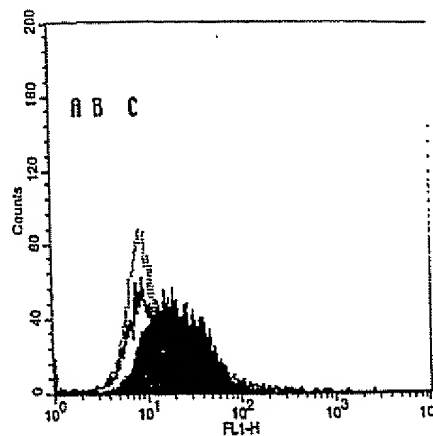
RAJI cells



A: unstained cells  
B: Rabbit sera 1532 control  
C: 1ug Rank-L + R1532  
D: 450ngs APRIL + R1532

B

RAJI cells



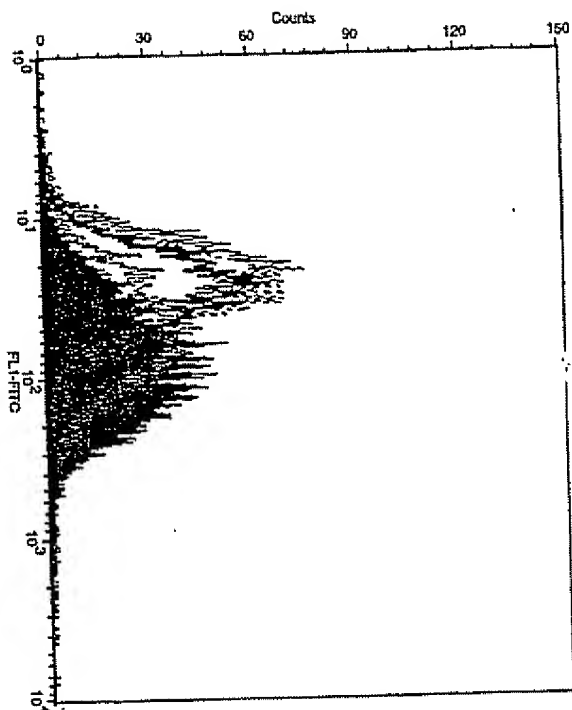
A: 450ngs APRIL + irrelevant rabbit sera  
B: Rabbit sera1532 control  
C: 450ngs APRIL + R1532



Figure 6

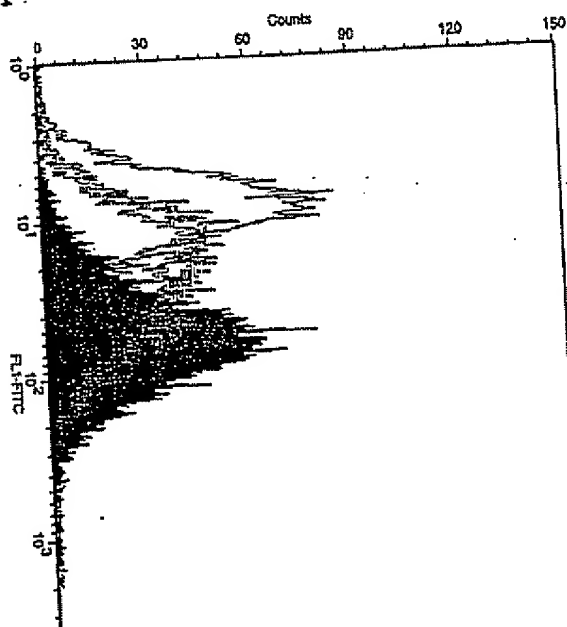
FIGURE 6. Competition for APRIL binding to B cell lines

A. A20 cells



BLACK: R1532 control  
GREEN: APRIL binding (450ngs)  
RED: APRIL + human BAFF (1ug)  
BLUE: APRIL + murin  
ORANGE: APRIL + BCI

B. RAJI cells



BLACK: R1532 control  
GREEN: APRIL binding (450ngs)  
RED: APRIL + human BAFF (1ug)  
BLUE: APRIL + BCMA-Ig (1ug)



figure 7a

A

A20 cells

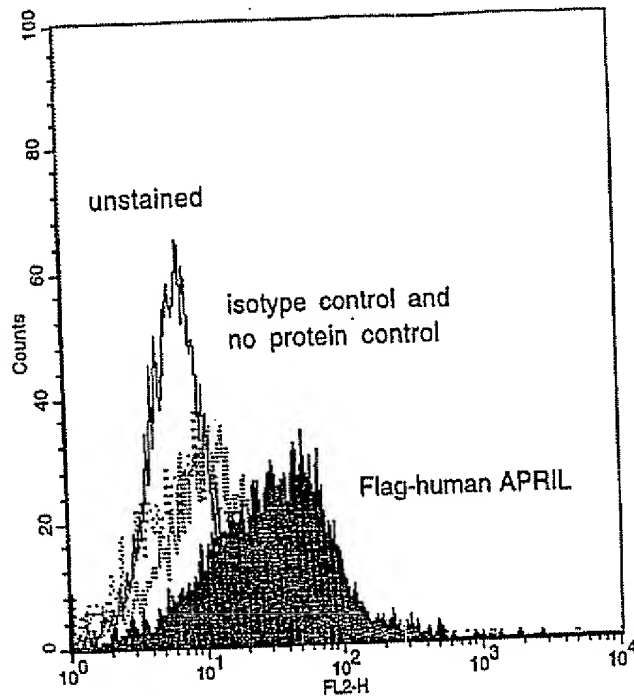


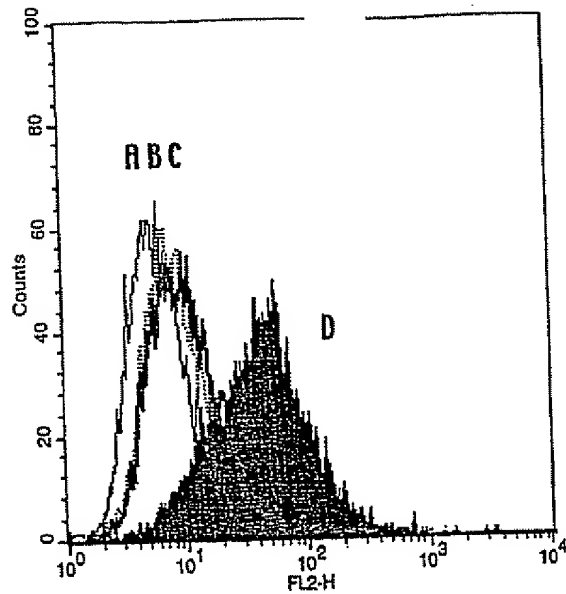




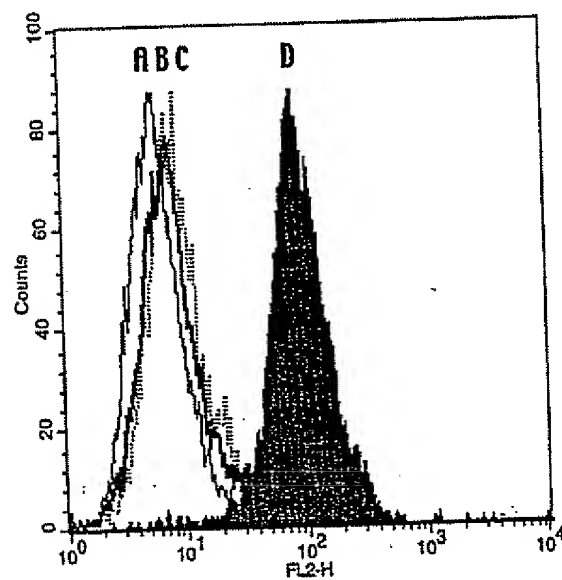
figure 7b

B

HT29 cells



HT29 cells



- A: unstained cells
- B: no protein control + biotinylated M2 anti-FLAG mAb
- C: 1ug FLAG-APRIL + biotinylated isotype control mAb
- D: 1ug FLAG-APRIL + biotinylated M2 anti-FLAG mAb

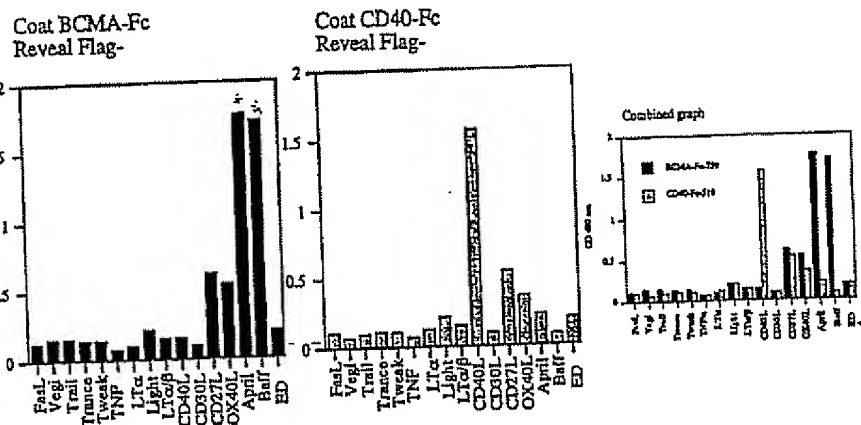
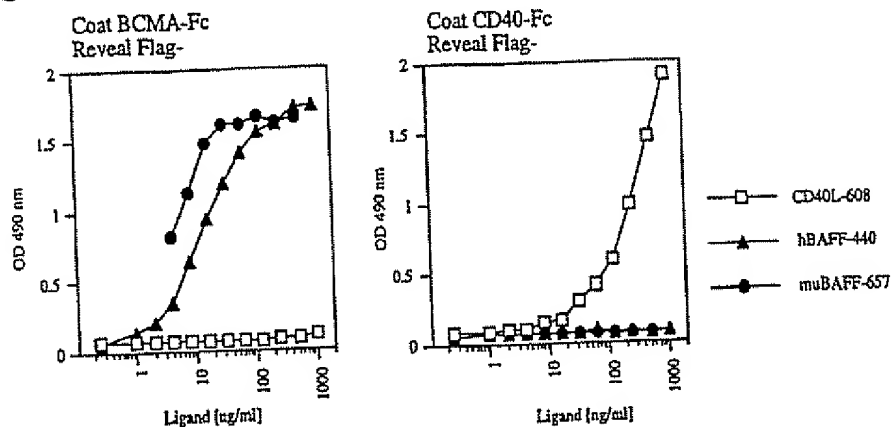






figure 7 A - hBaff-1 and hBaff-2 bind hBCMA-Fc  
in an ELISA format.

Specificity of BCMA-Fc. 24.08.99

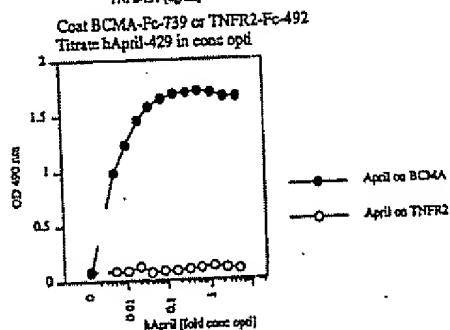
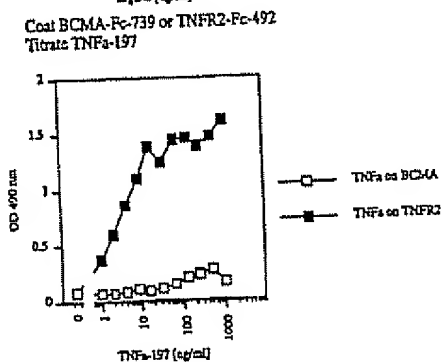
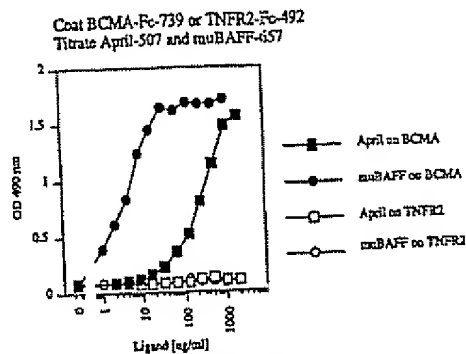


Coat plate with 100µl of 1µg/ml Rec-Fc in carbonate buffer (BCMA-Fc-739 or CD40-Fc-510).  
 Block with 5% milk in PBS 0.5% Tween-20.  
 Add ligand at indicated conc in milk (hBAFF-440, muBAFF-657, hCD40L-608) or as optimum  
 sups  
 (FasL-167, VegL-598, Trail-242, trance-545, Tweak-288, THFa-193, LTa-645, Light-555, Flag-  
 LTb-643/HA-LTa-644, CD40L-481, CD30L-647, CD27L-540, OX40L-525, April-429, Baff-336,  
 EDA-548). All have been checked for the presence of Flag-ligand by WB.  
 Incubate M2 at 1 µg/ml in milk (100µl)



# Figure 9 IS: ELISA dose responses.

April binds to BCMA-Fc in an ELISA test 26.8.99



Coat Rec-Fc (hBCMA-Fc-739 or hTNFR2-Fc-492) at 1 µg/ml in carbonate pH9.6. Block in PBS 5% milk, 0.5% Tween-20. Serial 2x dilutions of ligands in 100 µl of 10x dil milk (TNFa-197 1st final 1000ng/ml. muBAFF-657 1st final 1000ng/ml. hApril-507 1st final 2000 ng/ml (has gone through acid elution. Loss of activity?). hApril-429 in conc Optumem. 1st final conc is 5x conc optimum).

M2 in 10x dil milk (100 µl at 0.5 µg/ml)

Anti-mouse-PO 1/2000 in 10x dil milk (100µl). Reveal OPD.

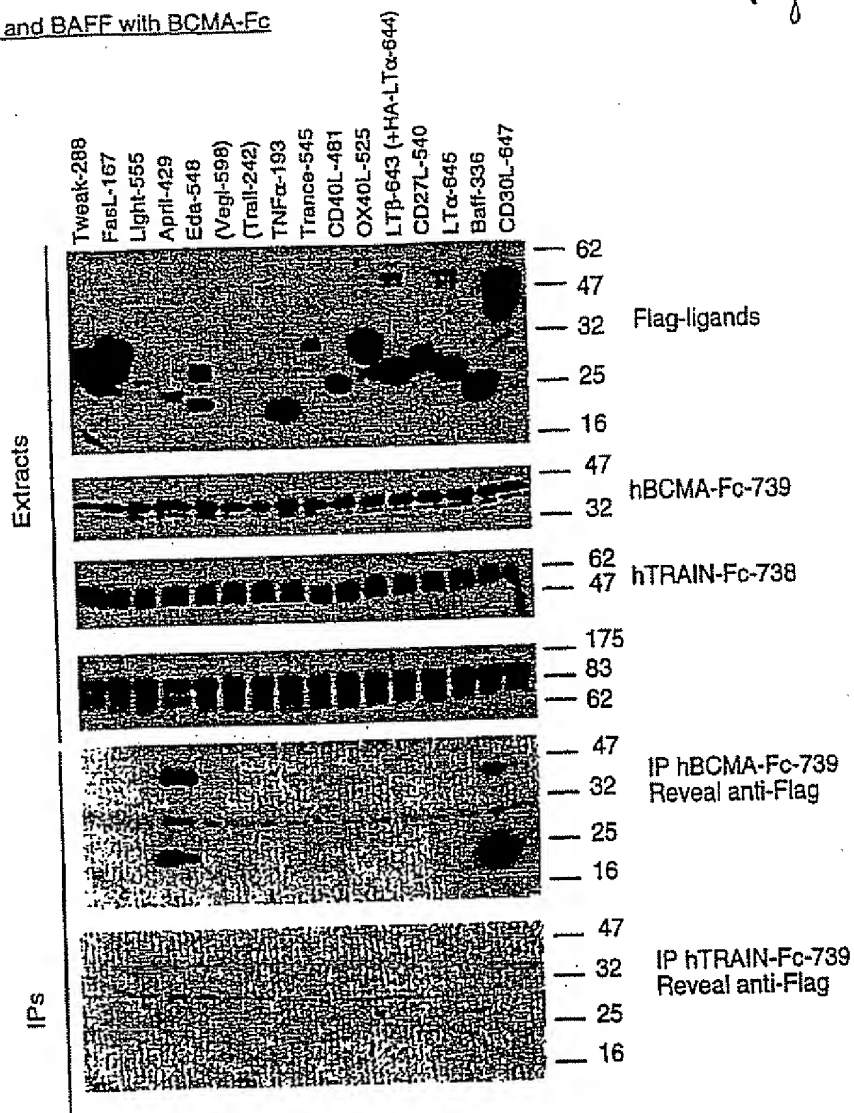
Note: Slight binding of TNF to BCMA is probably due to spill over of adjacent April-429 during the first wash step (I do it with a pisselle).





IP of April and BAFF with BCMA-Fc  
02.09.99

Figure 10



Transfect 293T with indicated expression plasmid (Rec-Fc or Flag ligand) in 9 cm plate.  
Wash, leave for 5d in 8 ml optimem.  
Mix 200  $\mu$ l of optimem receptor + 200  $\mu$ l of optimem ligand + 400  $\mu$ l PBS + 10  $\mu$ l ProtG-Sepharose.  
Leave 1 h on a wheel. Wash 4x with 1ml PBS. Boil in 50  $\mu$ l sample buffer (+DTT). Load 20  $\mu$ l.  
Reveal blot with M2 (1  $\mu$ g/ml) and anti-mouse-PO (1/2000). Reprobe blot with anti-human-PO.  
Ligands: Take 100  $\mu$ l optimem. Precipitate MeOH/CHCl<sub>3</sub>/lysozyme. Boil in 50  $\mu$ l sample buffer (+DTT). Load 20  $\mu$ l. Reveal blot with M2 (1  $\mu$ g/ml) and anti-mouse-PO (1/2000).



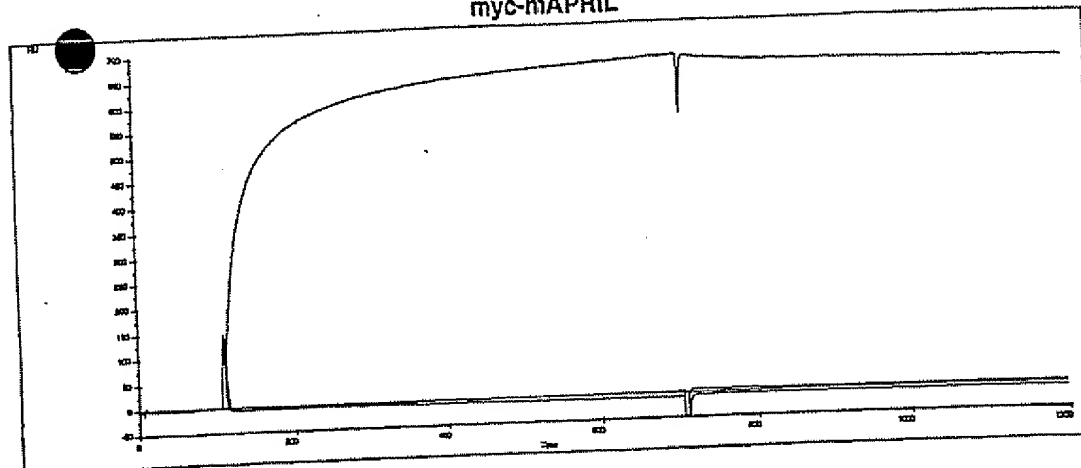
1= Blank

2= hBCMA

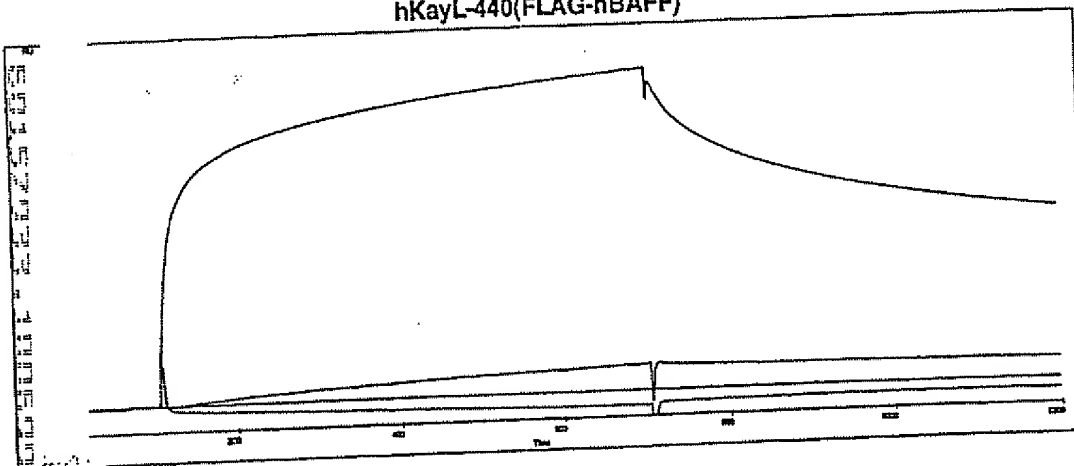
3= hLT $\beta$ R05

4= hp80 TNFR

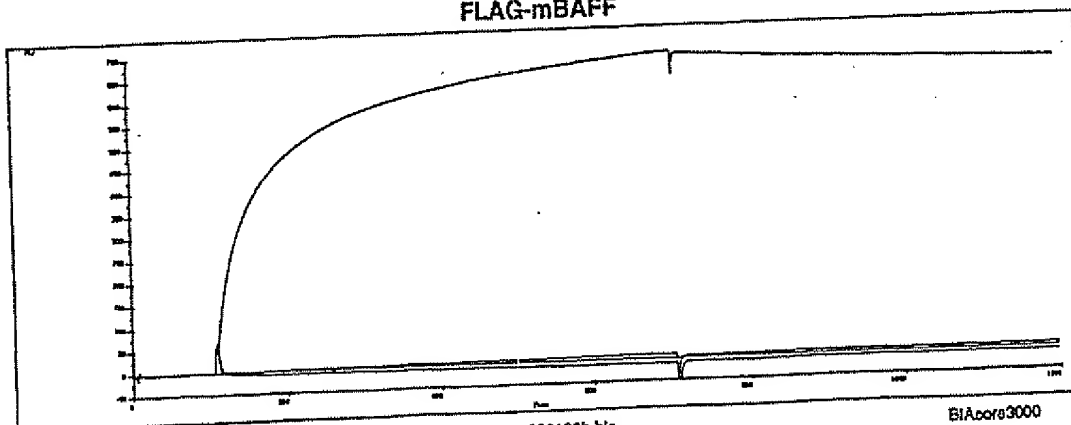
myc-mAPRIL



hKayL-440(FLAG-hBAFF)



FLAG-mBAFF

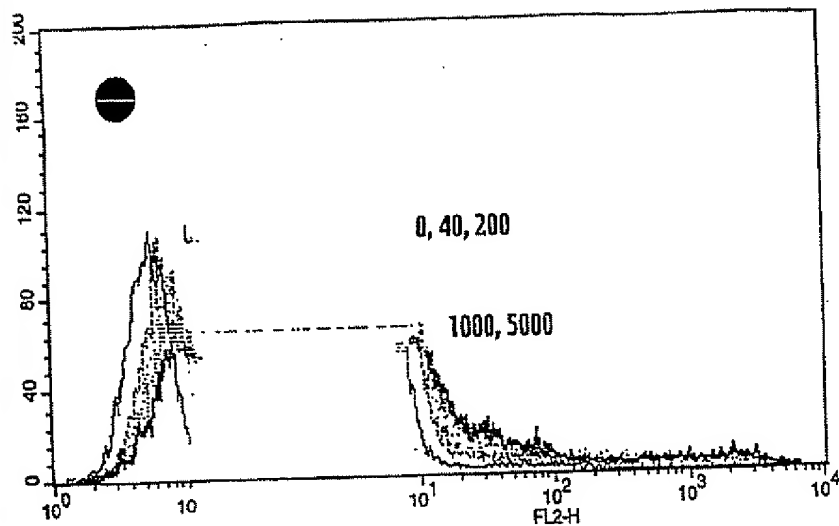


mz

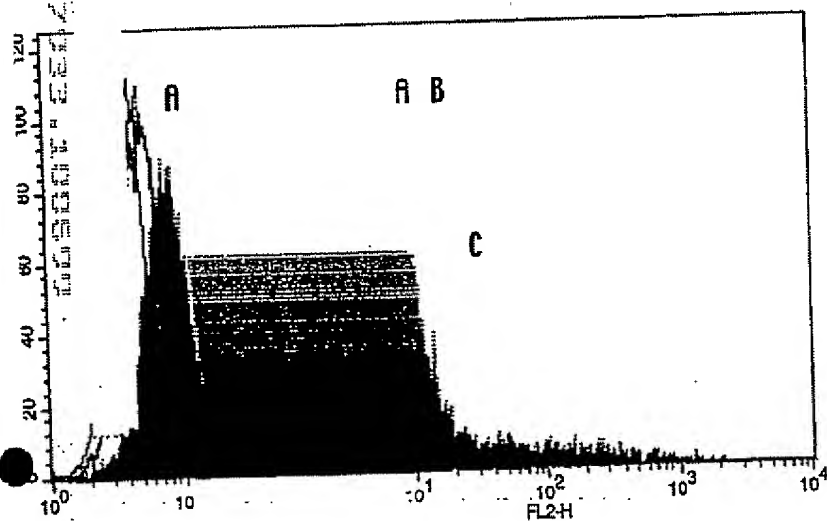
090189b.bla

BIAcore3000





93EBNA cells transfected with full length hBCMA were stained with 0, 40, 200, 1000, or 5000 ng/ml myc-mAPRIL, rabbit anti-APRIL antisera (R1532), and PE-labelled donkey anti-rabbit IgG.



293EBNA cells transfected with full length hBCMA:

- A: Rabbit sera (R1532) control
- B: 200 ng/ml myc-mAPRIL + 1 ug/ml BCMA-Ig
- C: 200 ng/ml myc-mAPRIL



